REMARKS

Claims 1-12 were pending in the application. Claims 1-12 are canceled without prejudice. Applicants reserve the right to prosecute the subject matter of claim 1-12 in one or more related continuation, divisional, and/or continuation-in-part applications. New claims 13-38 have been added. Support for the new claims can be found in the specification as set forth in the chart below.

Claim	Support (paragraph numbers in US 2002/0061513)
13	0006; 0010; 0015-0018; 0025; 0029-0031; 0037-38; 0048
14	0029
15	0029
16	0033
17	0025
18	0047
19	0012
20	0044
21	0033
22	0012; 0033; 0044; 0047
23	0012; 0033; 0044; 0047; 0029; 0060-0066
24	0006; 0028
25	0062
26	0024
27	0010
28	0057
29	0047
30, 31	0029
32, 33, 35	0022
34	0027
36, 37	0019
38	0011

None of the claim amendments comprises new matter. Thus, claims 13-38 will be pending upon entry of the present amendment.

The specification has been amended to include a specific reference to the prior application from which benefit of priority is claimed pursuant to 35 U.S.C. § 120. The claim for priority has been included in the oath or declaration submitted concurrently with the application on February 16, 2001, and the claim for priority has been recognized by the U.S. Patent and Trademark Office in the Filing Receipt mailed July 13, 2001. Thus, although the present amendment is being presented after the expiration of the time period required by 37 C.F.R. § 1.78(a)(2)(ii), a petition under 37 C.F.R. § 1.78(a) and the surcharge under 37 C.F.R. § 1.17(t) are not required, pursuant to M.P.E.P. Section 201.11(III)(D).

The Invention

The invention relates to a method for the purification of a polypeptide of interest. In particular, the polypeptide of interest is tagged with at least two different affinity tags, and the resulting fusion protein is expressed under conditions that allow association of the polypeptide of interest with other biomolecules to form a complex. Subsequently, the fusion protein is affinity purified using the two different affinity tags under conditions under which any complex that may have been formed between the polypeptide of interest and other biomolecules is maintained. Thus, the fusion protein is used as a bait to purify biomolecule complexes and their components with which the polypeptide of interest is associated.

The Rejection Under 35 U.S.C. § 112, First Paragraph, Based on Lack of Enablement Should Be Withdrawn

Claims 1-12 are rejected under 35 U.S.C. § 112, first paragraph, for alleged lack of enablement. In particular, it is argued that the claimed method is not enabled because undue experimentation would be required to identify the heterologous nucleic acids that would result in the desired complexes. It is further argued that the claimed method is only enabled for the purification and detection of complexes from yeast, and that the claimed method is only enabled for fusion proteins with the CBP-TEV-Protein A double tag wherein the fusion

protein is a protein complex with 24 subunits. The enablement rejection is also based on the allegation that the specification is not enabling for the claimed method because it fails to disclose in sufficient detail the conditions under which the complexes can be purified and detected. Applicants respectfully disagree. Applicants' arguments and responses to the rejection, to the extent that the rejection is relevant to the new claims, are set forth in detail below.

THE LEGAL STANDARD

The test for enablement is whether one reasonably skilled in the art could make or use the invention, without undue experimentation, from the disclosure in the patent specification coupled with information known in the art at the time the patent application was filed. *U.S. v. Telectronics Inc.*, 857 F.2d 778, 8 USPQ2d 1217 (Fed. Cir. 1988). In fact, well known subject matter is preferably omitted. See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384 (Fed. Cir. 1986) ("a patent need not teach, and preferably omits, what is well known in the art."). Further, one skilled in the art is presumed to use the information available to him in attempting to make or use the claimed invention. See *Northern Telecom, Inc. v. Datapoint Corp.*, 908 F.2d 931, 941 (Fed. Cir. 1990) ("A decision on the issue of enablement requires determination of whether a person skilled in the pertinent art, using the knowledge available to such a person and the disclosure in the patent document, could make and use the invention without undue experimentation."). These enablement rules preclude the need for the patent applicant to "set forth every minute detail regarding the invention." *Phillips Petroleum Co. v. United States Steel Corp.*, 673 F. Supp. 1278, 1291 (D. Del. 1991); see also *DeGeorge v. Bernier*, 768 F.2d 1318, 1323 (Fed. Cir. 1985).

Undue experimentation is experimentation that would require a level of ingenuity beyond what is expected from one of ordinary skill in the field. *Fields v. Conover*, 170 USPQ 276, 279 (CCPA 1971). The factors that can be considered in determining whether an amount of experimentation is undue have been listed in *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Among these factors are: the amount of effort involved, the guidance provided by the specification, the presence of working examples, the amount of pertinent literature and the level of skill in the art. The test for undue experimentation is not merely

quantitative, since a considerable amount of experimentation is permissible, so long as it is merely routine. *Id.*

Further, while the predictability of the art can be considered in determining whether an amount of experimentation is undue, mere unpredictability of the result of an experiment is not a consideration. Indeed, the Court of Custom and Patent Appeals has specifically cautioned that the unpredictability of the result of an experiment is not a basis to conclude that the amount of experimentation is undue in *In re Angstadt*, 190 USPQ 214 (CCPA 1976), at 218-219:

> [If to fulfill the requirements of 112, first paragraph, an applicant's] disclosure must provide guidance which will enable one skilled in the art to determine, with reasonable certainty before performing the reaction whether the claimed product will be obtained, ... then all "experimentation" is "undue" since the term "experimentation" implies that the success of the particular activity is uncertain. Such a proposition is contrary to the basic policy of the Patent Act. *Id.* at 219.

Thus, all that is required is a reasonable amount of guidance with respect to the direction of the experimentation; reasonable certainty with regard to the outcome of the experimentation is not required.

In addition, the Patent and Trademark Office bears the initial burden of establishing a *prima facie* case of non-enablement. *In re Marzocchi*, 169 USPQ 367, 369 (C.C.P.A. 1971); M.P.E.P. § 2164.02. A patent applicant's specification which contains a teaching of how to make and use the invention must be taken as enabling unless there is reason to doubt the objective truth of the teachings which must be relied on for enabling support. *Id.*

The Claimed Methods Are Enabled

The Examiner's attention is directed to the enclosed Declaration of Dr. Ulrich Kruse under 37 C.F.R. § 1.132 ("Kruse Declaration"), which presents evidence that the claimed method can be applied throughout its breadth without undue experimentation. Particularly, the Kruse Declaration demonstrates that the present specification in view of what was commonly known in the art is enabled for (i) the application of the claimed method to polypeptides of interest in general; (ii) the application of the claimed method to eukaryotic expression systems in general; and (iii) the use of the claimed method with affinity tags other

than the CBP-TEV-Protein A double tag that was used in Example 1 of the present application (see Kruse Declaration, at ¶4).

The claimed method can be practiced by following several distinct steps: (i) generation of the fusion protein from a polypeptide of interest; (ii) expression of the fusion protein; and (iii) purification of the fusion protein (see Kruse Declaration, at ¶5). Guidance for these individual steps can be found in the present application as follows: paragraphs 0007; 0030 to 0034; 0044 to 0048; and 0051[1] of the present specification for the generation of a fusion protein; paragraphs 0022 to 0025; 0027; and 0054 of the present specification for the expression of the fusion protein; and paragraphs 0028 to 0029 of the present specification for the purification of the fusion protein (see Kruse Declaration, at ¶5). This guidance in the application in combination with what was commonly known in the art at the time of filing of the application allows the skilled artisan to practice the claimed invention using merely routine experimentation (see Kruse Declaration, at ¶5).

Generation of the Fusion Protein

Preliminarily, Applicants respectfully point out that the new claims no longer recite "one or more heterologous nucleic acids." Instead, the claimed method requires that a fusion protein is expressed and that the fusion protein comprises a polypeptide of interest fused to at least two different affinity tags.

The claimed method is applicable to cellular polypeptides in general. As explained by Dr. Kruse, by the late 1990's, it had become apparent that "nearly every major process in a cell is carried out by assemblies of 10 or more protein molecules" (see Kruse Declaration, at ¶6, quoting Alberts, 1998, Cell 92:291-294, at p. 291, left col.; attached as Exhibit 2 to the Kruse Declaration; citing also Gavin et al., 2002, Nature 415:141-147; "Gavin;" attached as Exhibit 3 to the Kruse Declaration). Thus,

> [b]ecause of the vast numbers of polypeptides in eukaryotic cells that are expected to be components of protein assemblies in the cell, it is

[1] Citations to the present specification are to the application as published under publication number US 2002/0061513.

> not necessary to test whether the polypeptide of interest is part of a biomolecule complex before applying the TAP method. Rather, the skilled scientist could select a polypeptide of his or her choice and follow the procedure laid out and claimed in the '793 application, with a high probability of success in detecting and purifying the biomolecule complex with which the polypeptide of interest is associated, when such polypeptide is present in a complex in a eukaryotic expression environment. If the polypeptide does not participate in a complex in a eukaryotic expression environment, the method will purify the polypeptide alone.

See Kruse Declaration, at ¶6.

As illustrated in Gavin et al. (2002, Nature 415:141-147; "Gavin;" attached as Exhibit A), the presently claimed method can be used to characterize multiprotein complexes in a large scale, proteome-wide, approach:

> Gavin (Exhibit 3) applied the TAP technology to 1,739 open reading frames, *i.e.*, 1,739 polypeptides of interest, to gain insight in protein-protein interactions on a proteome level. Starting with these 1,739 open reading frames, Gavin et al. purified 589 tagged fusion proteins, 78% of which were found to be associated in complexes, with 232 non-redundant protein complexes being identified. The success rates of the individual steps: generation of the fusion proteins, expression of the fusion proteins, TAP purification, and identification of the complexes are shown in Figure 1c at page 142 of Gavin. Remarkably, despite the high throughput approach, the success rate for each step is far above 50%. Thus, Gavin has demonstrated that the TAP technology is generally applicable to polypeptides, and that the TAP technology can be routinely applied to different polypeptides without specific adjustment of the TAP method to each individual polypeptide of interest.

See Kruse Declaration, at ¶7. Thus, no undue experimentation would be necessary to apply the claimed methods to other polypeptides. Similarly, no undue experimentation would be required to select the affinity tags, and optionally intervening cleavage sites, for the generation of a fusion protein:

> In view of common knowledge in the fields of molecular biology and/or biochemistry in August 1998 and the description in the '793 application, the selection of suitable affinity tags would have been routine. The '793 application describes that the polypeptide of interest is fused to at least two different affinity tags. Several exemplary affinity tags are listed in the '793 application at paragraph 0007; many more affinity tags that can be used routinely were well-known in the art. In fact, affinity tags, their binding partners, and methods for purification of proteins using these affinity tags

were standard techniques in August of 1998 (see, *e.g.*, Section 4 entitled "Protein Recognition Tags Based on Affinity Interactions," at pp. 10 to 17 of Jones *et al.*, 1995, J. Chromatography A 707:3-22; "Jones;" attached hereto as Exhibit 4). The '793 application teaches at paragraph 0038 that the purification steps are chosen such that biomolecule complexes are maintained. In August 1998, it was well-known to a skilled scientist that certain buffer conditions, such as for example high or low pH, presence of chaotropic agents, or harsh detergents, would lead to dissociation of biomolecule complexes, and that buffers that tended to simulate physiological conditions (*e.g.*, of pH and salt concentration) would be preferred. The types of molecular interactions affecting the stability of individual proteins and the association of proteins with other proteins were well-known (hydrogen bonds, hydrophobic interactions, and ionic interactions); also well-known were conditions (*e.g.*, based on temperature, salt concentration, pH values) that would preserve these interactions (see, *e.g.*, Marshak et al., 1996. Strategies for Protein Purification and Characterization. A Laboratory Course Manual. Cold Spring Harbor Laboratory Press 1996, Introduction, pp. 1 to 10; attached as Exhibit 5). Accordingly, the skilled artisan would have chosen affinity tags that allow elution from the affinity matrix under conditions that would not dissociate the biomolecule complexes (*e.g.*, elution with chelating agent; see paragraph 0044 of the '793 application). Alternatively or additionally, the skilled scientist could separate the affinity tag from the polypeptide of interest by a cleavage site to allow release of the polypeptide (or the truncated fusion protein) as described at paragraphs 0029-0033 and 0048 of the '793 application. Such cleavage sites were also well-known to a skilled scientist. For example, Jones (Exhibit 4, in particular the paragraph spanning pp. 11 and 12), discusses cleavage sites that can be used to separate a polypeptide of interest from an affinity tag. Thus, it is my opinion that the skilled scientist could select and use various suitable combinations of affinity tags and cleavage site(s) to purify a particular biomolecule complex in a straightforward manner based on the teachings of the '793 application and standard knowledge in the field.

See Kruse Declaration, at ¶8. Thus, one skilled in the art could readily employ affinity tags other than the CBP-TEV-ProteinA double tag used in Example 1 of the instant application.

Once the polypeptide of interest and the affinity tags are selected, the nucleic acid encoding the fusion protein can be cloned using standard cloning techniques:

> It is also my judgment that creating a recombinant nucleic acid containing a nucleotide sequence that encodes the polypeptide of interest fused to two or more affinity tags with one or more optional cleavage sites intervening between the encoded tag and the encoded polypeptide could be performed using standard cloning techniques that were routine in August 1998. With regard to the location of the affinity tags, the '793 application teaches that the affinity tags can be fused at the N- or C-terminus of the polypeptide, or

> even internally in the polypeptide (paragraph 0047). Commonly known vectors and molecular cloning techniques that can be used, for example, are described in Sambrook et al. (1989, 2nd Edition, Strategies for Cloning in Plasmid Vectors, pp. 1.53 to 1.73, In: Molecular Cloning—A Laboratory Manual, Cold Spring Harbor Laboratory Press; attached as Exhibit 6), which is a standard textbook that is cited at paragraph 0050 of the '793 application.

See Kruse Declaration, at ¶9. Thus, the skilled artisan could, based on the disclosure in the present specification and the common knowledge in the art, generate fusion proteins to be used in the claimed methods without undue experimentation.

Expression of the Fusion Protein

If the fusion protein is expressed in a cell, several additional steps can be part of the claimed method:

> As taught in the '793 application, where the skilled scientist chooses to express the fusion protein in a recombinant host cell, the following individual steps can also be part of the TAP method: (a) transfection of the nucleic acid that encodes the fusion protein into a host cell (paragraph 0023 of the '793 application); (b) expression of the nucleic acid in the host cell (paragraphs 0021 to 0025; and 0027 of the '793 application); and (c) extraction of proteins from the cell (paragraphs 0006 and 0025 of the '793 application).

See Kruse Declaration, at ¶10. Based on the teachings in the present specification, and what was commonly known in the art, the skilled artisan could have practiced these steps wihout undue experimentation (see Kruse Declaration at ¶¶10 to 12). With regard to the transfection step, Dr. Kruse states in the Kruse Declaration:

> Transfection of nucleotide sequences into cells was also a very well established, routine technique in August 1998. Protocols for the transfection of nucleotide sequences into different cell types such as insect cells, yeast cells, and mammalian cells were standard in molecular biology laboratories at that time. For example, Sambrook et al. (1989, 2nd Edition, Introduction of Recombinant Vectors into Mammalian Cells, pp. 16.30 to 16.72, In: Molecular Cloning—A Laboratory Manual, Cold Spring Harbor Laboratory Press; attached as Exhibit 7) provides protocols for transfection of DNA into mammalian cells.

See Kruse Declaration, at ¶10. With respect to the expression of nucleic in host cells or in cell-free systems, Dr. Kruse states in the Kruse Declaration:

> Similarly, numerous protocols for the expression of a nucleotide sequence in a eukaryotic expression system were readily available to a skilled scientist in August 1998. Appropriate promoters for the expression system of choice were also standard tools. For example, Sambrook et al. (1989, 2nd Edition, Functional Components of Mammalian Expression Vectors, pp. 16.5 to 16.29, In: Molecular Cloning—A Laboratory Manual, Cold Spring Harbor Laboratory Press; attached as Exhibit 8) discusses promoters, enhancer sequences, and polyadenylation sites that can be used for recombinant expression of proteins in mammalian cells. Further, an overview of protein expression in mammalian cells is also provided in Ausubel et al. ((Editors), 1995, Unit 16.12: Overview of Protein Expression in Mammalian Cells, In: Short Protocols in Molecular Biology, Third Edition, John Wiley & Sons, Inc., attached hereto as Exhibit 9). The '793 application discloses at paragraph 0050, consistent with what was well known in the art of molecular biology and/or biochemistry, that the expression of the fusion protein is regulated by control sequences, such as promoters, enhancers, and poly-A sites. The '793 application also provides a working example using expression in yeast cells (see paragraphs 0060 to 0069). Furnished with the teachings of the '793 application and the general knowledge in the field of molecular biology in August 1998, a skilled scientist could have routinely selected suitable control sequences, vectors, and host cells for the expression of the fusion protein in eukaryotic cells. Alternatively, use of a cell-free system to achieve a eukaryotic expression environment was also well-known in the art (see, *e.g.*, King et al., 1997, Science 277:973-974; attached hereto as Exhibit 10; see also paragraph 0027 of the '793 application).

See Kruse Declaration, at ¶11. Finally, with respect to the extraction of proteins from the host cell in which they are expressed, Dr. Kruse states in the Kruse Decaration:

> Obtaining protein extracts from cells and manipulation of cell extracts under conditions that leave many protein and other biomolecule complexes intact has been a standard technique in biology laboratories for a long time, and was well-established by August 1998. For example, Smith (May 1998, Methods 15(1):27-39; attached hereto as Exhibit 11) published a protocol for the preparation of cell extracts in order to study RNA editing protein complexes assembled on Apolipoprotein A mRNA. This extraction protocol was successfully applied to diverse cell types such as McArdle 7777 rat hepatoma, HepG2 human hepatoma, HeLa human cervical carcinoma, Chinese hamster ovary (CHO), and a variety of other mammalian cells (see especially pp. 30-32 of Exhibit 11). As discussed above, conditions that would not dissociate biomolecule complexes were well-known to a skilled scientist. In view of the state of the art and the direction in the '793 application to maintain the biomolecule complexes (paragraph 0038), a skilled scientist would have been taught, and able routinely, to avoid harsh conditions that would be expected to dissociate biomolecule complexes.

See Kruse Declaration, at ¶12. Thus, a fusion protein that is to be used with the claimed method can be expressed in a host cell and the appropriate protein extracts prepared from the host cell by the skilled artisan without undue experimentation.

Affinity Purification of the Fusion Protein

Further, Dr. Kruse states that "guidance for the affinity purification in the '793 application and the state of the art of affinity purification in August 1998 are discussed above in paragraph 8 [of the Kruse Declaration], showing that only routine skill would be expected to be needed in the implementation of those steps" (see Kruse Declaration, at ¶13).

In fact, affinity purification protocols were so standard even years before the earliest priority date, that Jones et al., 1995, in their review article, which is cited at paragraph 8 of the Kruse Declaration and attached as Exhibit 4 thereto, state that "[affinity-tag-based purification] reduces the time needed to develop new purification schemes for new proteins and simplifies the purification process allowing individuals, *not skilled in the art*, to successfully purify their proteins without the need of extensive protein purification expertise" (emphasis added; p. 12, right col., section 4.1.2, of Jones et al., 1995, J Chromatography A 707:3-22).

Successful Application to Eukaryotic Expression Systems

The claimed methods have been succussfully applied to various eukaryotic expression environments (see Kruse Declaration, at ¶¶14 and 21). "Example 1 in the '793 application and Gavin (Exhibit 3) provide evidence that the TAP method can be successfully used for polypeptides in yeast" (see Kruse Declaration, at ¶14).

> Additional evidence demonstrates that the TAP method is applicable to other eukaryotic cells, such as mammalian cells. For example, Cox et al. (2002, Biotechniques 33:267-270; "Cox;" attached hereto as Exhibit 12) shows the successful application of the TAP method in a mammalian expression system. Following the steps of the TAP method, Cox purified a protein complex from mammalian cells. For each individual step of the TAP method, a skilled scientist, using merely routine experimentation,

> could have adopted the guidance of the '793 application and arrived at the specific conditions used in Cox.

See Kruse Declaration, at ¶14.

Cox selected the affinity tags and intervening cleavage sites in accordance with the teachings of the present application without undue experimentation:

> Cox used an N-terminal TAP affinity tag. Cox's fusion protein included the following elements in the following order starting at the N-terminus: two IgG binding domains of *Staphylococcus aureus* protein A ("ProtA"), TEV protease cleavage site ("TEV"), calmodulin binding site ("CBP"), enterokinase cleavage site ("EK"), and Cox's polypeptide of interest (MEF2-A). [footnote omitted] Apart from the second cleavage site (EK), Cox used the same CBP-TEV-ProtA double tag that is described in the '793 application (see paragraph 0048). In fact, Cox obtained the TAP tag vectors from Dr. Seraphin, an inventor of the '793 application (see Acknowledgment at page 269 of Cox). That a second cleavage site can be part of the TAP tag is disclosed in the '793 application at paragraph 0048.

See Kruse Declaration, at ¶15. Similarly, Cox used a commonly known expression system and transfection procedures to express the fusion protein:

> Cox used a commercially available expression vector with a cytomegalovirus (CMV) promoter and tetracycline-regulated control sequence. Both regulatory sequences were commonly known and routinely used by skilled scientists in August 1998 for expression of a protein of interest in a mammalian cell (see, *e.g.*, Holwell et al., 1997, J Cell Science 110:1947-1956; attached hereto as Exhibit 14; see also Sambrook (Exhibit 8)). Many other promoters that can be used for expression in mammalian cells such as the COS7 cells used by Cox were well-known in August 1998 (see, *e.g.*, Sambrook (Exhibit 8)). The TAP tag, *i.e.*, ProtA-TEV-CBP-EK and the coding region for MEF2-A were cloned into the cloning vector using standard molecular cloning techniques.

See Kruse Declaration, at ¶16.

> Cox transfected the expression vector into the mammalian COS7 cell line using calcium phosphate precipitation. Calcium phosphate precipitation was a standard technique to transfect eukaryotic cells with DNA as of August 1998 (see, *e.g.*, Exhibit 7).

See Kruse Declaration, at ¶ 17. Protein extraction from the host cells was also performed using routinely available techniques:

> Protein extract was obtained from the transfected cells in Cox by lysing the cells by freeze/thaw cycles. After lysis, Nonidet P-40 (a nonionic detergent) was added. Both the use of NP-40 and freeze/thaw cycles were routine in the art (see, *e.g.*, Sambrook et al. (1989, 2nd Edition, Immunoprecipitation, pp. 18.26 to 18.33, In: Molecular Cloning—A Laboratory Manual, Cold Spring Harbor Laboratory Press; attached as Exhibit 15; and Chapter 16 Expression of Cloned Genes in Cultured Mammalian Cells, pp. 16.59 to 16.62; attached as Exhibit 16), and are not harsh conditions that generally dissociate protein-protein complexes. Since the '793 application teaches that protein complexes, where present, are to be isolated (see, *e.g.*, paragraph 0038 of the '793 application), it is my opinion that a skilled scientist would know to choose conditions such as these that minimize the dissociation of protein-protein interactions.

See Kruse Declaration, at ¶18. And lastly, with respect to the affinity purification, no undue experimentation would have been necessary to apply the teachings of the present specification to Cox's polypeptide of interest:

> Cox also used routine experimentation and methods taught in the '793 application to conduct the affinity purification steps. Both the ProtA and the CBP affinity purifications used by Cox are taught in the '793 application, and commonly known in the art (see, *e.g.*, Jones (Exhibit 4)). Again, since protein complexes were to be purified, a skilled scientist would have been aware that conditions that generally would dissociate biomolecule complexes are to be avoided.

See Kruse Declaration, at ¶19.

> Using procedures as described in the '793 application in Example 1 (paragraphs 0060 to 0068), Cox purified the complex via binding to a support, cleavage with Tobacco Etch Virus protease NIA, binding to a support, and elution, all using essentially the same method as described in the '793 application, thereby evidencing the straightforward applicability of the TAP method to mammalian cells.

See Kruse Declaration, at ¶20.

In view of what was commonly known in the art, the skilled artisan could have simply followed the guidance set forth in the present application and purified biomolecule complexes as described by Cox without undue experimentation (see Kruse Declaration, at ¶¶15 to 20).

Another publication evidences the enablement by the specification of eukaryotic expression systems generally. As stated by Dr. Kruse: "Further evidence of the straightforward applicability of the TAP method to mammalian cells is found in Ju et al."

(2004, Cell 119:815-829, attached as Exhibit 17 to the Kruse Declaration; see Kruse Declaration, at ¶21). "Ju provides another example of the application of the TAP method to mammalian cells, specifically to 293 cells. In the Experimental Procedures section, Ju relies on a reference to Puig (Exhibit 13), which essentially describes the TAP method as taught in the '793 application" (see Kruse Declaration, at ¶21).

Conclusion

The foregoing leads Dr. Kruse to conclude that:

> in August 1998, a skilled scientist, using only routine experimentation, would have been able (i) to apply the claimed methods of the '793 application for purifying polypeptides, or biomolecule complexes containing polypeptides, from a eukaryotic expression system in general, such as a mammalian or yeast system; (ii) to apply these methods to nucleic acids encoding different polypeptides of interest; and (iii) to implement these methods using different affinity tags.

See Kruse Declaration, at ¶22. Thus, the claims are enabled.

The Kruse Declaration provides evidence that the claimed methods are enabled. The rejections should thus be withdrawn. In the event the Examiner disagrees, and to the extent that this rejection is based on facts within her personal knowledge, Applicants request that the Examiner provide an affidavit pursuant to the provisions of 37 C.F.R. 1.104(d)(2).

The Rejection Under 35 U.S.C. § 112, First Paragraph, Based on Lack of Written Description Should Be Withdrawn

Claims 1-12 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly not being supported by sufficient written description. The written description rejection is premised on the allegation that Applicants were not in possession of the heterologous nucleic acids that would result in the formation of biomolecule complexes because the specification allegedly fails to disclose specific heterologous nucleic acids and because the specification allegedly further fails to provide evidence that a heterologous nucleic acid, upon its expression, forms a biomolecule complex. Applicants respectfully disagree as set forth in detail below.

THE LEGAL STANDARD

The test for sufficiency of written description is whether the disclosure of the application 'reasonably conveys to the artisan that the inventor had possession' of the claimed subject matter. *In re Kaslow*, 707 F.2d 1366, 1375, 217 U.S.P.Q. (BNA) 1089, 1096 (Fed. Cir. 1983); accord *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563; *see also*, *Ralston Purina Co. v. Far-Mar-Co, Inc.*, 772 F.2d 1570, 1575, 227 U.S.P.Q. (BNA) 177, 179 (Fed. Cir. 1985). The Court of Appeals for the Federal Circuit has repeatedly considered the written description requirement and consistently found that exacting detail is not necessary to meet the requirement:

> If a person of ordinary skill in the art would have understood the inventor to have been in possession of the claimed invention at the time of filing, even if [not] every nuance of the claims is explicitly described in the specification, the adequate written description requirement is met. *In re Alton*, 76 F.3d 1168, 37 USPQ2d 1578 (Fed. Cir. 1996).

The criteria for determining sufficiency of written description set forth in Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112 ¶ 1, "Written Description Requirement" ("the Guidelines") (published in the January 5, 2001 Federal Register at Volume 66, Number 4, p. 1099-1111), specifies that:

> The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice (see (1)(a) above), reduction to drawings (see (1) (b) above), or by disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus (see (1)(c), above). *Id.* at p. 1106, column 3, *l.* 13-29.

Where the specification discloses any relevant identifying characteristics, *i.e.*, physical, chemical and/or functional characteristics sufficient to allow a skilled artisan to recognize the applicant was in possession of the claimed invention, a rejection for lack of written description under Section 112, first paragraph, is misplaced. *Id.*

Furthermore, in accordance with the Guidelines, what is conventional or well known to one of skill in the art need not be disclosed in detail (*Id.* at p. 1105, column 3, *ll.* 39-41),

and, where the level of knowledge and skill in the art is high, a written description question should not be raised. *Id.* at p. 1106, column 1, *ll.* 34-36. See also *Capon v. Eshhar*, 418 F.3d 1349, at 1357 (Fed. Cir. 2005).

The Claimed Methods Are Sufficiently Described

The new claims no longer recite "one or more heterologous nucleic acids." Instead, the claims require that a polypeptide of interest is expressed as a fusion protein, wherein the fusion protein compises the polypeptide of interest and at least two different affinity tags. As discussed above, and as illustrated by Gavin on a proteome-wide scale, the fusion protein serves as a bait to purify any biomolecule complex with which the polypeptide of interest is associated. Thus, the present method is generally applicable to polypeptides to purify biomolecule complexes of which the polypeptide of interest is a component.

Firstly, Applicants note that the claimed methods do not require that a biomolecule complex be isolated. If the polypeptide of interest does not participate in the formation of a biomolecule complex in a eukaryotic expression environment, the method will purify the polypeptide alone (see Kruse Declaration, at ¶6).

Applicants assert that they have disclosed sufficient chemical characteristics for a sufficient written description of the polypeptide of interest. Since polypeptides generally can be used with the present method, all that is required in terms of structure is that the polypeptide of interest is a polypeptide. Moreover, as explained by Dr. Kruse in the Kruse Declaration, "nearly every major process in a cell is carried out by assemblies of 10 or more protein molecules" (see Kruse Declaration, at ¶6, quoting: Alberts, 1998, Cell 92:291-294, attached as Exhibit 2 to the Kruse Declaration).

> Because of the vast numbers of polypeptides in eukaryotic cells that are expected to be components of protein assemblies in the cell, it is not necessary to test whether the polypeptide of interest is part of a biomolecule complex before applying the TAP method. Rather, the skilled scientist could select a polypeptide of his or her choice and follow the procedure laid out and claimed in the '793 application, with a high probability of success in detecting and purifying the biomolecule complex with which the polypeptide of interest is associated, when such polypeptide is present in a complex in a eukaryotic expression environment."

Kruse Declaration at ¶6. Thus, the claimed method is generally applicable to polypeptides.

With regard to the Examiner's argument that the specification fails to disclose specific heterologous sequences, Applicants respectfully submit that the present case is analogous to that of *Capon*. In *Capon*, the Court of Appeals for the Federal Circuit ("Federal Circuit") vacated a decision by the United States Patent and Trademark Office Board of Patent Appeals and Interferences ("Board") that claims directed to chimeric DNA molecules failed to meet the written description requirement. The chimeric DNA molecules were composed of (i) a fragment encoding a single chain antibody; fused to (ii) a fragment encoding a part of a lymphocyte signaling protein. The Board had hald that the "nucleotide sequences of the chimeric genes must be fully presented." *Id.* at 1358. The Federal Circuit held that "the descriptive text needed to meet [the written description requirement] varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence," and rejected the Board's holding because the state of the scientific knowledge must be considered and because precedents do not "require a re-description of what was already known" to satisfy the written description requirement. *Id.* at 1357. In particular, the Federal Circuit explained that, because the invention does not concern the discovery of gene function or structure, and rather concerns novel chimeric genes prepared from DNA segments of known structure and function, the specification need not recite specific sequences in order to satisfy the written description requirement. *Id.* at 1358. Similarly, in the present case, nucleotide sequences of polypeptides and of affinity tags were known at the time of filing of the application, and the invention does not concern the discovery of the sequences of the polypeptide of interest or affinity tags. As discussed above and in the Kruse Declaration, the present specification provides sufficient guidance to combine a polypeptide with two or more affinity tags to practice the currently claimed invention. Thus, the rejection should be withdrawn.

The Rejection Under 35 U.S.C. § 102 Should Be Withdrawn

Claims 1-9 and 11-12 are rejected as allegedly anticipated under 35 U.S.C. § 102(b) by Darzins et al. (WO 96/40943; "Darzins"). Applicants assert that the rejection over Darzins does not apply to the new claims for the reasons set forth below.

The Legal Standard

The standard for an anticipatory reference is set forth in *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987): "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *See also Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236 (Fed. Cir. 1989)(holding that "[t]he identical invention must be shown in as complete detail as is contained in the . . . claim").

It is well established that in order for a prior art reference to amount to an inherent anticipation of a claim, all the elements of the claim must necessarily, inevitably, and always result from the prior art disclosure and would be so recognized by one of ordinary skill in the art; mere possibilities or probabilities are not sufficient. *See* Continental Can Co. USA v. Monsanto Co., 948 F.2d 1264, 1269, 20 U.S.P.Q.2d 1746, 1749 (Fed. Cir. 1991); W.L. Gore & Assocs., Inc. v. Garlock, Inc., 721 F.2d 1540, 1553-54, 220 U.S.P.Q. 303, 313-14 (Fed. Cir. 1983), cert. denied, 469 U.S. 851 (1984); In re Oelrich, 666 F.2d 578, 581, 212 U.S.P.Q. 323, 325-26 (C.C.P.A. 1981); Phillips Petroleum Co. v. U.S. Steel Corp., 673 F.Supp. 1278, 1295 n.12, 6 U.S.P.Q.2d 1065, 1076-77 n.12 (D. Del. 1987), aff'd, 865 F.2d 1247, 9 U.S.P.Q.2d 1461 (Fed. Cir. 1989); Hughes Aircraft Co. v. U.S., 8 U.S.P.Q.2d 1580, 1583 (Ct. Cl. 1988); Ex parte Levy, 17 U.S.P.Q.2d 1461, 1463-64 (B.P.A.I. 1990); Ex parte Skinner, 2 U.S.P.Q.2d 1788, 1788-89 (B.P.A.I. 1987). As stated by the Court of Appeals for the Federal Circuit:

> we are not persuaded that the 'effect' of the processes disclosed in [the prior art patents], an 'effect' undisclosed in those patents, would be always to inherently produce or be seen always to produce products meeting all of the claim limitations. Anticipation of inventions set forth in product claims cannot be predicated on mere conjecture respecting the characteristics of products that might result from the practice of processes disclosed in references.

W.L. Gore & Assocs., Inc. v. Garlock, Inc., 721 F.2d 1540, 1554, 220 U.S.P.Q. 303, 314 (Fed. Cir. 1983), cert. denied, 469 U.S. 851 (1984) (citing In re Felton, 484 F.2d 495, 500, 179 U.S.P.Q. 295, 298 (C.C.P.A. 1973)).

As has also been stated:

> Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing *may* result from a given set of circumstances is not sufficient.

In re Oelrich, 666 F.2d 578, 581, 212 U.S.P.Q. 323, 326 (C.C.P.A. 1981) (citing Hansgirg v. Kemmer, 102 F.2d 212, 214, 40 U.S.P.Q. 665, 667 (C.C.P.A. 1939)).

And as stated by the U.S. Court of Claims:

> The Williams claim is not anticipated under the doctrine of inherency. . . . It does not flow undeniably and irrefutably from the express disclosures of [the prior art reference] whether anyone recognizes it or not . . . The [prior art reference] does not expressly or inherently disclose each element of the Williams claim and therefore does not anticipate it.

Hughes Aircraft Co. v. U.S., 8 U.S.P.Q.2d 1580, 1583 (Ct. Cl. 1988) (emphasis added).

Thus, it is not sufficient that a teaching of a prior art reference could yield a result that would anticipate the claim against which the prior art reference is applied; instead, to be anticipatory under the doctrine of inherency, the teaching of the prior art reference must inevitably lead to the result.

Darzins Fails to Teach Each and Every Claim Element

The new claims require that the fusion protein comprising the polypeptide of interest be expressed in a eukaryotic expression environment. Darzins, however, is concerned with protein expression in Gram-positive bacteria and is consequently devoid of any description of eukaryotic expression systems. In fact, Darzins' invention "is based on the finding that many gram-positive bacteria sort proteins to their cell surface through *cis*-acting N-terminal signal sequences and C-terminal anchor regions" (see Abstract of Darzin). Darzins discloses a method wherein a protein of interest is fused to the *cis*-acting N-terminal signal sequences and C-terminal anchor regions and expressed in a Gram-positive host. Once expressed the fusion protein of interest is anchored via a covalent bond to the bacterial cell wall. The fusion protein of interest is subsequently cleaved off the cell wall and purified. Since binding to the bacterial cell wall is an integral step of Darzins invention, eukaryotic expression systems are not described in this reference. Thus, at least for this reason, Darzins does not anticipate the claimed invention.

Darzins Fails to Explicitly Disclose Conditions that Allow Formation and Preservation of a Biomolecule Complexes

The claimed method specifies that any of the other biomolecules which may be bound to the polypeptide of interest remain associated with the polypeptide during the

purification so as to permit the detection and/or purification of biomolecule complexes. In contrast, Darzins is concerned with the production and purification of recombinant proteins (Darzins, at page 8, lines 7-9). An explicit teaching that conditions should be used under which a biomolecule complex would be preserved during expression and purification of the protein is absent from Darzins. Thus, for this additional reason, Darzins does not explicitly anticipate the claimed invention.

Darzins Fails to Inherently Disclose Conditions that Allow Formation and Preservation of Biomolecule Complexes

Many different purification schemes are listed in Darzins at page 15, line 17 through page 16, line 2. Although various purification methods are listed in Darzins, Darzins is silent as to the precise conditions under which the purification procedures should be conducted, and thus the methods taught by Darzins do not inevitably preserve biomolecule complexes. In particular, Darzins does not teach that conditions that would dissociate biomolecule complexes, such as the presence of harsh detergents, high or low pH, and presence of chaotropic agents, are to be avoided. As another example of why the disclosed methods do not inevitably preserve biomolecule complexes, if dialysis were chosen to purify the polypeptide of interest and the pore size for the dialysis were such that only the polypeptide of interest but not a biomolecule complex could pass through the pores, any biomolecule complexes comprising the polypeptide of interest would not be recovered. Since Darzins is silent as to the specific purification conditions, it cannot be said that the teachings in Darzins will necessarily and inevitably lead to conditions that allow the preservation of biomolecule complexes. Consequently, following the teachings of Darzins does not necessarily and inevitably lead to an expression environment and purification scheme under conditions that allow formation and preservation, respectively, of biomolecule complexes, and thus Darzins does not inherently disclose this feature of the claimed invention. For this additional reason, Darzins does not anticipate the claimed invention.

The Rejection Under 35 U.S.C. § 103 Should Be Withdrawn

Claim 10 is rejected as allegedly being obvious under 35 U.S.C. § 103(a) over Darzins in view of Zheng *et al.* (1997, Gene 186:55-60; "Zheng"). Claim 10 (now canceled) specified that one of the affinity tags consists of at least one calmodulin binding peptide ("CBP"). The use of CBP as an affinity tag is not described in Darzins. Zheng was cited by

the Examiner as disclosing CBP fusion proteins and the use of the CBP affinity tag in affinity purification. Applicants assert that this rejection does not apply to the new claims, and in particular, it does not apply to new claims 20, 22, 23, and claims dependent thereon, which specify the use of of one or more calmodulin binding peptides.

The Legal Standard

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the prior art references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. M.P.E.P. 2143.

Prior art references may be combined to render an invention obvious under 35 U.S.C. § 103, however, the teachings of references can be combined only if there is some suggestion or incentive to do so. *ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1575 (Fed. Cir. 1984). The teaching or motivation to combine prior art references must be "clear and particular. Broad conclusory statements regarding the teaching of multiple references, standing alone, are not evidence." *In re Dembiczak*, 173 F.3d 994, 999 (Fed. Cir. 1999).

The Federal Circuit has expressly indicated that a *prima facie* case of obviousness requires "objective evidence of record" demonstrating that there is prior art that teaches or suggests combining the asserted references as proposed. *In re Lee*, 277 F.3d 1338, 1341 (Fed. Cir. 2002). More specifically, the motivation to combine references originate from one of three sources: the nature of the problem to be solved, the teachings of the prior art, or the knowledge of persons of ordinary skill in the art. *In re Rouffet*, 47 USPQ2d 1453, 1457-58 (Fed. Cir. 1998). Consequently, the reason or suggestion in the art for carrying out the invention, must originate from a source other than the knowledge learned from the Applicant's disclosure (*In re Dow Chemical Co.*, 837 F.2d 469, 473 (Fed. Cir. 1988)), and care must be exercised not to use the Applicant's disclosure to fill in the gaps in the prior art. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991); *In re Grabiak*, 769 F.2d 729 (Fed. Cir. 1985).

Further, an obviousness rejection cannot be based on inherent disclosure in a prior art reference. The Court of Customs and Patent Appeals stated "the inherency of an advantage and its obviousness are entirely different questions. That which may be inherent is not

necessarily known. Obviousness cannot be predicated on what is unknown." *In re Spormann*, 363 F.2d 444, 448 (C.C.P.A. 1966).

Darzins and Zheng Fail to Teach Each and Every Claim Element

As discussed above, Darzins does not teach expression in a eukaryotic expression environment, but instead is limited to bacterial expression systems. Darzins discloses a method wherein a protein of interest is fused to the *cis*-acting N-terminal signal sequences and C-terminal anchor regions and expressed in a Gram-positive host. Once expressed, the fusion protein of interest is anchored via a covalent bond to the bacterial cell wall. The fusion protein of interest is subsequently cleaved off the cell wall and purified. Since binding to the bacterial cell wall is an integral step of Darzins invention, eukaryotic expression systems would be inoperable with Darzins method and thus are clearly unsuitable for use in Darzins method. Thus, there is no hint or suggestion of the use of eukaryotic expression environments in Darzins. Zheng does not rectify this deficiency, since Zheng provides no suggestion to apply Darzins' method to eukaryotic expression environments. Zheng is concerned with the expression of fusion proteins with the calmodulin-binding peptide as an affinity tag in *E. coli*. Thus, neither Darzins nor Zheng teach a eukaryotic expression environment in a method as claimed.

Further, neither Darzins (see discussion above) nor Zheng suggests that expression conditions under which a biomolecule complex can be formed, and purification conditions under which the complex would be preserved, should be used.

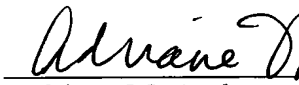
In view of the foregoing, the rejection under 35 U.S.C. § 103(a) over Darzins in view of Zheng should be withdrawn.

CONCLUSION

Applicants respectfully request that the amendments and remarks of the present response be entered and made of record in the instant application. Withdrawal of the Examiner's rejections and an allowance of the application are earnestly requested. If any issues remain in connection herewith, the Examiner is respectfully invited to telephone the undersigned to discuss the same.

Respectfully submitted,

Date January 17, 2006



32,605

Adriane M. Antler (Reg. No.)
JONES DAY
222 East 41st Street
New York, New York 10017
Telephone Number 212-326-3939